

Attorney Docket No.: RU-0124  
Inventors: Breslauer et al.  
Serial No.: 09/869,004  
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This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of the claims:

Claims 1-49 (canceled)

Claim 50: (new) A method for screening for nucleic acid duplex stability by competitive equilibria comprising:

(a) producing a solution containing a known amount of an initial nucleic acid duplex with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to a target strand and labeled with a donor of a FET pair and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand and labeled with an acceptor of the FET pair;

(b) titrating the solution with a second solution comprising a known concentration of the target nucleic acid strand which competes with the first nucleic acid strand of the initial nucleic acid duplex of step (a) for binding to the second nucleic acid strand of the initial nucleic acid duplex of step (a), said target nucleic acid strand being

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single- or double-stranded;

(c) subjecting the titrated solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the target strand and the second nucleic acid strand of the initial nucleic acid duplex of step (a) upon titration in step (b), but which do not disrupt the target strand when double-stranded;

(d) subjecting the titrated solution to conditions which promote duplex or triplex formation; and

(e) monitoring the titrated solution for changes in the amount of initial nucleic acid duplex formed as a function of the amount of target nucleic acid strand added by measuring changes in FET donor or acceptor intensity.

Claim 51: (new) A method for screening for nucleic acid duplex stability comprising:

(a) producing a solution containing an initial nucleic acid duplex with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand labeled with a donor of a FET pair and a second nucleic acid strand

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labeled with an acceptor of the FET pair, each strand being capable of forming a duplex with a double-stranded target strand;

(b) titrating the double-stranded target strand into the solution;

(c) subjecting the titrated solution to conditions which disrupt the initial nucleic acid duplex of step (a), the double-stranded target strand, and any duplex between the disrupted target strands and the first and second nucleic acid strands of the initial nucleic acid duplex of step (a);

(d) subjecting the titrated solution to conditions which promote duplex formation; and

(e) monitoring the titrated solution for changes in the amount of initial nucleic acid duplex formed as a function of the amount of double-stranded target nucleic acid strand added by measuring changes in FET donor or acceptor intensity.

Claim 52: (new) A method for detecting a single

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nucleotide polymorphism comprising:

(a) producing an initial nucleic acid duplex comprising a first and second nucleic acid strand, wherein the first or second strand of the initial nucleic acid duplex is designed to identify a single nucleotide polymorphism in a single- or double-stranded target nucleic acid sequence and wherein the first nucleic acid strand comprises a donor nucleic acid strand labeled with a donor of a FET pair and the second nucleic acid strand comprises an acceptor nucleic acid strand labeled with an acceptor of the FET pair;

(b) measuring FET donor or acceptor intensity indicative of the amount of the initial nucleic acid duplex produced in step (a);

(c) adding a fixed excess amount of the single- or double-stranded target nucleic acid strand into the solution;

(d) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex or triplex formed between the single- or double-stranded target strand and the first or second nucleic acid strand of

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the initial nucleic acid duplex of step (a) upon addition of the single- or double-stranded target strand in step (c), but which do not disrupt the target strand when double-stranded;

(e) subjecting the titrated solution to conditions which promote duplex or triplex formation; and

(f) measuring FET donor or acceptor intensity indicative of the amount of initial nucleic acid duplex formed after addition of the single- or double-stranded target strand wherein the measured amount after addition of the single- or double-stranded target strand is indicative of the single- or double-stranded target strand containing the single nucleotide polymorphism.

Claim 53: (new) A method for detecting a single nucleotide polymorphism comprising:

(a) producing an initial nucleic acid duplex comprising a first and second nucleic acid strand, wherein the first or second strand of the duplex is designed to identify a single nucleotide polymorphisms in a double-stranded target nucleic

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acid sequence and wherein the first nucleic acid strand comprises a donor nucleic acid strand labeled with a donor of a FET pair and the second nucleic acid strand comprises an acceptor nucleic acid strand labeled with an acceptor of the FET pair;

(b) measuring FET donor or acceptor intensity indicative of the amount of the initial nucleic acid duplex;

(c) adding a fixed excess amount of the double-stranded target nucleic acid strand into the solution;

(d) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a), the double-stranded target nucleic acid sequence and any duplex formed between the double-stranded target strand and the first or second nucleic acid strand of the initial nucleic acid duplex of step (a) formed upon addition of the double-stranded target strand in step (c);

(e) subjecting the titrated solution to conditions which promote duplex formation; and

(f) measuring FET donor or acceptor intensity

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indicative of the amount of initial duplex formed after addition of the target strand wherein the measured amount after addition of the target strand is indicative of the target strand containing the single nucleotide polymorphism.

54. (new) A method for determining the concentration of a target nucleic acid sequence comprising:

(a) adding a known volume and concentration of an initial nucleic acid duplex with a known stability to a known volume of a solution containing a target strand, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target strand and labeled with a donor of a FET pair and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand and labeled with an acceptor of the FET pair;

(b) subjecting the solution to conditions which disrupt the initial nucleic acid duplex of step (a) and any duplex between the target strand and the first nucleic acid strand or the second nucleic acid strand of the initial nucleic

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acid duplex of step (a);

(c) subjecting the solution to conditions which promote duplex formation; and

(d) determining the relative change in the amount of initial nucleic acid duplex formed in the solution by measuring changes in FET donor or acceptor intensity.

Claim 55: (new) A method for determining the concentration of a target nucleic acid sequence comprising:

(a) adding a known volume of a solution of target strand to a known volume of a solution containing a known concentration of an initial nucleic acid duplex with a known stability, said initial nucleic acid duplex comprising a first nucleic acid strand having a sequence wholly or in part homologous to the target strand and labeled with a donor of a FET pair and a second nucleic acid strand having a sequence wholly or in part complementary to the target strand and labeled with an acceptor of the FET pair;

(b) subjecting the solution to conditions which disrupt the initial nucleic acid duplex and any duplex between the



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target strand and the first or second nucleic acid strand of the initial nucleic acid duplex;

(c) subjecting the solution to conditions which promote duplex formation; and

(d) determining the relative change in the amount of initial nucleic acid duplex formed in the solution by measuring changes in FET donor or acceptor intensity.

Claim 56: (new) A method for assessing stability of various selected target strands comprising:

(a) selecting various target strands;

(b) performing the method of claim 1 with the same initial nucleic acid duplex and each of the selected target strands; and

(c) comparing monitored changes in the amount of initial nucleic acid duplex formed as a function of the amount of the selected target nucleic acid strand added by measuring changes in FET donor or acceptor intensity to ascertain differences in stability of duplexes or triplexes formed by the various target strands.